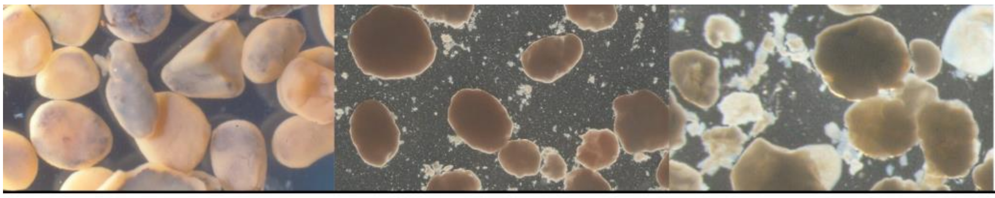

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Anaerobic granulation for bioproduction: High rate production of medium chain carboxylic acids from thin stillage

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Abstract: Medium chain carboxylic acids can be generated by fermentation. In current literature, high rate production of caproic and caprylic acids is subject to addition of exogenous ethanol and lactate as electron donors for chain elongation, online product extraction or operation at circumneutral pH values. Here we demonstrate the feasibility of high rate chain elongation by promoting biomass aggregation in a reactor fed with the liquid fraction with thin stillage at low pH. We achieved a maximum MCCA production rate of 30.6 g L^{-1} d^{-1} at pH 5.5. The granules were disc shaped which we hypothesize facilitates the diffusion of toxic products out of the biofilm.

Keywords: caproic acid; fermentative granules; UASB

Session 8 – *Application of granular sludge technologies*

Introduction

Caproic (n-hexanoic) and caprylic (n-octanoic) acids are medium chain carboxylic acids (MCCAs) that have been targeted as carbon-resource recovery products under the carboxylate platform (Agler *et al.* 2011). Their value, far beyond that of methane, stems in their wide variety of applications as additives in personal care products, plasticizers or functional feed products for livestock production, among others. Fermentative production of MCCAs was first reported in the early 1940s with lactic acid or ethanol as electron donors (Barker & Taha 1942; Barker *et al.* 1945). The microbial process, commonly referred to as chain elongation, occurs by reversed β-oxidation and results in the elongation of short chain carboxylic acids (electron acceptor) with 2 carbons at a time (from the electron donor). Only in the recent years, this microbial process has been investigated for bioproduction, initially in synthetic media, using ethanol as electron donor (Steinbusch *et al.* 2011) and later with complex organic feedstocks, often rich in ethanol or lactate(Agler *et al.* 2012; Andersen *et al.* 2017; Khor *et al.* 2017).

High MCCA production rates (up to 57.4 g MCCA L^{-1} d^{-1}) have been observed in bioreactors fed with exogenous ethanol at circumneutral pH (Grootscholten *et al.* 2013). This operating conditions require the addition of expensive exogenous ethanol and the relatively high pH needs vast amounts of base and acid for pH control and product extraction, respectively, increasing the overall cost and carbon footprint of the process (Chen *et al.* 2017). However, high rates of MCCAs production rate have not been obtained when fermenting organic wastes or industrial side products at a low pH. When the feed is devoid in lactate and ethanol, the chain elongation process occurs in concomitance with hydrolysis, primary fermentation and generation of suitable electron donors, and the low pH enhances the toxic effect of the fermentation products leading to low specific rates. Therefore, high volumetric rates are subject to improved biomass retention.

Uncoupling hydraulic residence time (HRT) from solid retention time (SRT) can be performed with a physical barrier, but filtration adds up to the overall capital and operation costs, and is problematic with solid rich effluents. On the other hand, promoting biomass aggregation into granules would enable achieving high biomass concentrations in the reactor, low solid concentration in the effluent and would allow the different microbial processes to occur within the close vicinity of the granule. Granular biomass has been widely applied for anaerobic wastewater treatment (Lettinga *et al.* 1980), nitrogen removal (van der Star *et al.* 2007) and aerobic wastewater treatment (Morgenroth *et al.* 1997). However, the literature about application of granular biomass for bioproduction is scarce, and only one report investigated the application of granular sludge for production of MCCAs (Roghair *et al.* 2016). Here we investigated the production of caproic acid in an upflow anaerobic fermenter (UAF) with granular biomass at pH 5.5 under different HRTs.

Material and Methods

The fermentation was run in a 1.6-L UAF in a temperature controlled room at 34±2 °C. The inoculum was obtained from the fermentation effluent of a pilot plant producing MCCA from full thin stillage. The UAF was continuously fed with the (solid-free) liquid fraction of thin stillage for 120 d. The pH was controlled at 5.5 with addition of NaOH 2-3M. The HRT was varied from 3.91-0.31 d. The solid-free stillage had a COD of 50.0±4.0 g L^{-1} and contained lactic acid (4.1±1.1g L^{-1}), acetic acid (1.0±0.5 g L^{-1}), glycerol (7.2±2.6 g L^{-1}) and ethanol (0.2±0.3 g L^{-1}). NH_4Cl was amended at 0.5 gN L^{-1}.

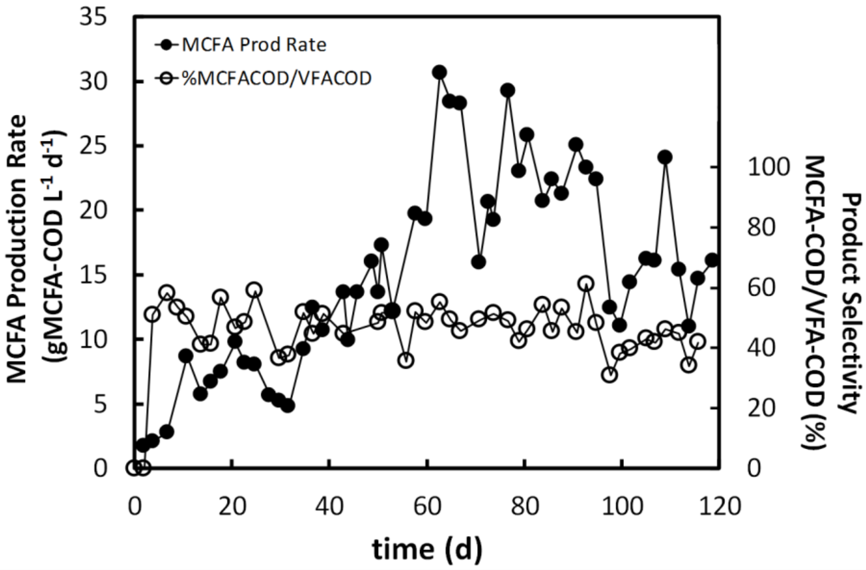
Results and Conclusions

The reactor was started up at a HRT of 3.91d. After 7d, the concentration of caproate reached 4.7 g L^{-1}. The loading rate was stepwise increased as the concentration of caproate reached 6 g L^{-1}, resulting in an HRT of 1.7d from days 9-28, 0.8d from days 29-53 and 0.6d from day 54 onwards. Granulation was only observed after the HRT was decreased from 1.7d to 0.8d. The granulation did not affect the product speciation. MCCA accounted for 54.1±4.2% of the VFA-COD produced from days 9-25 and 51.6±4.4% from day 50-120. Only from day 26-49 the fraction of COD recovered as MCCA fell down to 24.7% of the VFA-COD, which coincided with the halving of the HRT. The MCCA production rate peaked at 30.6 g COD L-1 d^{-1} (Fig. 1), and only fell to 12.4 g COD L^{-1} d^{-1} on day 100, after a change in feed batch. Caproate was the dominant MCCA, although caprylate and enanthate were also detected. The gas production and composition were measured regularly. Methane was never detected, indicating the absence of methanogens. The biogas was composed by H_2 and CO_2 (no H_2S was detected) at 45±5% and 55±5%, respectively, and H_2 production accounted for 6.1±1.8% of the fed COD between the days 50-120.

Lactate was almost always depleted in the effluent, except on days 14, 37 and 58, coinciding with a peak in caproate concentration, reactor overloading and pH probe malfunctioning, respectively. On the other hand the concentration of ethanol was always higher in the effluent than in the influent, reaching up to 2.7 g L^{-1} on day 16, but was lower after granulation started, indicating that ethanol could serve as electron donor for elongation.

The granules were initially spherical and turned disc shaped after a while (Figure 2). We hypothesise that the disc shape was caused by product toxicity, and facilitated the diffusion of the MCCA out of the granules. SEM imaging showed a variety of bacteria with different shapes including cocci, rod, bacilli and clostridium-like spore forming bacteria, embedded in a dense EPS matrix.

Molecular analysis of the microbial community is being performed as well as analysis of the dominant EPS of the biomass. Overall we show the first high rate system for production of caproic acid from a complex industrial sidestream, without exogenous addition of electron donors for elongation and at low pH.



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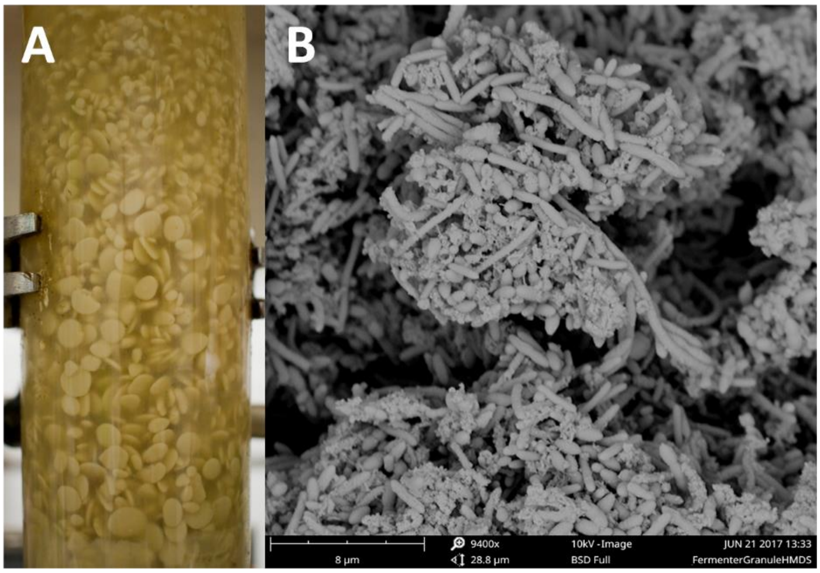

Figure 2 Close up picture of the fermentative granules in the UAF (A). SEM micrograph of a fermentative granule HMDS-dried and fixed in glutaraldehyde (4%) (B).

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